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ORIGINAL ARTICLE

Identification and validation of QTL for spike fertile floret and fruiting efficiencies in hexaploid wheat (*Triticum aestivum* L.)

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Abstract

Key message **This study identified and validated two QTL associated with spike fertile floret and fruiting efficiencies. They represent two new loci to use in MAS to improve wheat yield potential.**

Abstract The spike fruiting efficiency (FE—grains per unit spike dry weight at anthesis, GN/SDW) is a promising trait to improve wheat yield potential. It depends on fertile floret efficiency (fertile florets per unit SDW—FFE, FF/SDW) and grain set (grains per fertile floret—GST). Given its difficult measurement, it is often estimated as the grains per unit of nongrain spike dry weight at maturity (FEm). In this study, quantitative trait loci (QTL) were mapped using a double haploid population (Baguette 19/BIOINTA 2002, with high and low FE, respectively) genotyped with the iSelect 90 K SNP array and evaluated in five environments. We identified 37 QTL, but two were major with an $R^2 > 10\%$ and stable for being at least present in three environments: the *QFEm.perg-3A* (on Chr. 3A, 51.6 cM, 685.12 Mb) for FEm and the *QFFE.perg-5A* (on Chr. 5A, 42.1 cM, 461.49 Mb) for FFE, FE and FEm. Both QTL were validated using two independent F_2 populations and KASP markers. For the most promising QTL, *QFFE.perg-5A*, the presence of the allele for high FFE resulted in + 4% FF, + 9% GN, + 13% GST, + 16% yield gSDW^{-1} and + 5% yield spike^{-1}. *QFEm.perg-3A* and *QFFE.perg-5A* represent two new loci to use in MAS to improve wheat yield potential.

Abbreviations

B19	Baguette 19
B2002	BIOINTA 2002
CH	Chaff (no-grain spike dry weight at maturity, g spike^{-1})
CN	Compactness of the spike (mm node^{-1})
DH	Double haploid
E1 to E5	Testing environments
FFE	Fertile floret efficiency (florets $g_{SDW}{}^{-1}$)
FE	Fruiting efficiency (grains $g_{SDW}{}^{-1}$)
FEm	Fruiting efficiency at maturity (grains $g_{CH}{}^{-1}$)
FF	Fertile florets per spike (n° spike^{-1})
FFFS	Fertile florets per fertile spikelet (n° spikelet^{-1})
FS	Fertile spikelets per spike (n° spike^{-1})
GN	Grain number per spike (n° spike^{-1})
GST	Grain set (n° grains floret^{-1})
GW	Grain weight (g)

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Pop 1	F_2 population showing segregation for the *QFFE.perg-5A*
Pop 2	F_2 population showing segregation for the *QFEm.perg-3A*
SDW	Spike dry weight at anthesis (g spike^{-1})
SL	Spike length (mm)
TS	Total spikelets per spike (n° spike^{-1})

Introduction

Wheat (*Triticum aestivum* L.) is one of the main cereals that supply the world demand for food. Given that it provides 20% of the calories in the human diet, increasing current production would assist in securing proper food provision (Tweeten and Thompson 2008; Chand 2009; Reynolds et al. 2012). Improving cultivar's yield potential (i.e., the yield of an adapted cultivar without water or nutritional restrictions and free of biotic stresses, Evans 1996) is an alternative to increase current production (Fischer 1983; Calderini and Slafer 1998; Slafer and Araus 2007; Fischer 2007; Reynolds et al. 2009; Fischer and Edmeades 2010). Wheat breeding for yield potential has been based on the empirical selection of yield per se due to the complexity of the trait and the lack of knowledge and useful tools, either physiological and/or genetic, with actual applicability in the breeding programs (Snape and Moore 2007). Understanding the physiological and genetic basis of the attributes that define the yield potential in the target environment helps to identify promising attributes to assist traditional breeding (Slafer 2003).

Grain number per unit area and particularly grain number per spike (GN) were the main drivers of improved yield potential through breeding eras (Waddington et al. 1986; Perry and D'Antouno 1989; Siddique et al. 1989; Slafer and Andrade 1989; Slafer and Andrade 1993; Acreche et al. 2008; Del Pozo et al. 2014; Lo Valvo et al. 2018). GN is determined during the period taking place *ca.* 20 days before and 10 days after anthesis (Fischer 1975, 1985). During this period, around 40–60% of the floret primordia stop development (or die) reducing to a few those that reach the fertile stage at anthesis (Langer and Hanif 1973; Kirby 1988; Siddique et al. 1989; González et al. 2011a). The competition for assimilates between the stem and the growing spike would be the cause of the floral mortality during this period (Fischer 1975, 1985; Kirby 1988; Ghiglione et al. 2008; González et al. 2011a). After anthesis, the number of fertile florets that set grains is established (Fischer 1975, 1985). Based on this ecophysiological model, Fischer (1983) proposed to understand grain number as the product of the spike dry weight at anthesis (SDW) and the fruiting efficiency of the spike (number of grains achieved per unit dry weight of spike at anthesis—FE). As the SDW is complex to measure (it is highly time-consuming and destructive), the no-grain spike dry weight at maturity (or chaff—CH) is used as a surrogate to SDW. Then, the fruiting efficiency is often calculated at maturity (FEm) as the number of grains produced per unit of chaff dry weight. Nevertheless, as the relationship between the chaff and the SDW is extremely variable (Fischer and Stockman 1980; Stockman et al. 1983; Slafer et al. 2015; Elía et al. 2016; Pretini et al. 2020) by factors still unknown (Fischer 2011; Slafer et al. 2015), the correlation between FE and FEm may be high or low (or even negative), depending on environment and genetic population (Elía et al. 2016; Pretini et al. 2020). Although the correlation between FE and FEm has been questioned (Elía et al. 2016; Pretini et al. 2020), given the high correlation between grain number and both FE and its proxy FEm, the fruiting efficiency has been proposed as a trait to improve grain number and yield potential (Abbate et al. 1998; González et al. 2011b; Slafer et al. 2015). A recent study showed that selecting for FEm in early generations might help to improve yield in later generations (Alonso et al. 2018). Despite this promising report, the complex measurement of fruiting efficiency may limit its broad use in commercial breeding programs as secondary trait. The detection of QTL genetically linked with it would be the first step to facilitate selection.

In recent years, many publications have been generated regarding QTL for yield and associated traits in wheat (Quraishi et al. 2011; Chen et al. 2016, 2017; Deng et al. 2017; Cheng et al. 2017; Yu et al. 2018; Zhai et al. 2018; Xu et al. 2017). All of them identified numerical attributes of yield performance but not ecophysiological determinants. To our knowledge, three GWAS studies were conducted recently for FEm. The first one (Guo et al. 2017) identified a region on chromosome 2A using an association mapping population of European winter cultivars, which was later identified as the *GNI-A1* gene, associated with higher number of fertile florets and grains per spikelet (Sakuma et al. 2019). The second one (Gerard et al. 2019) detected four SNPs on chromosomes 2A, 2D, 4D and 5A using a small association panel formed with cultivars from 20 countries across five continents. Finally, for Argentinean wheat cultivars, Basile et al. (2019) detected 17 genome regions distributed on seven wheat chromosomes (1A, 2A, 3B, 4A, 5A, 6A and 7A). Furthermore, once a QTL is identified, its validation is desirable before using linked markers for MAS (Dao et al. 2017), which has not been done in the last two exploratory reports.

Studying the simpler physiological traits determining FE may help to identify simpler genetic bases for assisted selection. The FE is a complex trait itself, involving (1) the determination of fertile florets at anthesis, (2) the partitioning of dry matter within the spike (among florets-grains and no-grain spike) and (3) the grain set. Then, Fischer (2011) dissected the FE as the product of the number of fertile florets per unit of spike dry weight at anthesis (naming it fertile floret efficiency—FFE—in

the present study) and grain set (grains/fertile floret—GST). As far as we know, there are no previous reports trying to identify QTL for FE and FFE, not even to perform a validation study after identification, which would enable its actual use in MAS for breeding yield potential in wheat.

The number of fertile florets per spike would depend on the number of spikelets bearing florets (or fertile spikelets—FS) and the number of fertile florets per fertile spikelet (FFFS). Breeding yield potential during the green revolution increased the number of fertile florets per spikelet, with no impact on spikelets per spike (Siddique et al. 1989). There are no reports considering the FFE variation among a large set of genotypes associated with differences in these components.

The first aim of the study was to detect stable QTL for FFE, FE and FEm using a double haploid population specially designed to study fruiting efficiency. It consisted of 102 lines derived from the cross between two Argentinean wheat cultivars, Baguette 19 with high and BIOINTA 2002 with low FE and FEm according to González et al. (2011a, b) and Terrile et al. (2017) (Fig. 1). Once the QTL were identified, the second objective was to validate them through the development of independent F_2 populations and associating the phenotype with the peak markers transformed to Kompetitive allele-specific PCR (KASP) markers suitable for their use in MAS (Fig. 1). The third objective was to determine whether the validated QTL for fertile floret and fruiting efficiencies had pleiotropic effects not only on the final GN and its physiological determinants (FF and GST), but also on the spike structure traits (i.e., total spikelets—TS, fertile spikelets—FS, fertile floret per fertile spikelet—FFFS, spike length—SL and compactness—CN or spike length (mm) per rachis node) (Fig. 1). Finally, as some authors reported a negative relation between FE and the SDW (or FEm and CH at maturity) (Slafer et al. 2015; Terrile et al. 2017; Lo Valvo et al. 2018), or between FE and grain weight (GW) (Slafer et al. 2015; Terrile et al. 2017), the pleiotropic effects of the validated QTL were also tested for SDW, CH, GW, yield/SDW and yield.

We report in the present study the mapping of several QTL for FFE, FE and FEm. Two of these QTL were further validated in independent F_2 populations, one for FFE, FE and FEm located on the 5A chromosome and another one for FEm located on 3A chromosome.

Materials and methods

Plant materials and populations development

The QTL mapping was conducted using a population of 102 double haploid (DH) lines specially designed to study FE in the breeding target environment (Fig. 1). The DH population was developed through in vitro anther culture (De Buyser and Henry 1980) of the F_2 gametes generated from a cross between "Baguette 19/BIOINTA 2002." Baguette 19 (B19) (pedigree not available) is a semidwarf hard cultivar released by Nidera Semillas in 2006 in Argentina. BIOINTA 2002 (B2002) (BPON/CCTP-F7-7792–122(87)) is a semidwarf hard cultivar developed by CIMMYT (International Maize and Wheat Improvement Center), released in 2006 in Argentina by INTA. B19 and B2002 were identified in a previous screening as low and high FEm, respectively (González et al. 2011b), which was confirmed also for FE in a later study (Terrile et al. 2017) (Fig. 1). B19 and B2002 are both spring cultivars (B19: *Vrn-A1b/vrn-B1/vrn-D1; B2002: vrn-A1/Vrn-B1/vrn-D1*) and mostly insensitive to photoperiod (*Ppd-D1a*). Once the previous DH population was phenotyped and QTL identified, two independent F_2 populations of 500 plants each were generated to validate the two largest QTL, one for FFE, FE and FEm (*QFFE.perg-5A),* and the other for FEm (*QFEm.perg-3A)* (Fig. 1). Each population derived from the cross between two selected DH lines from B19/B2002 population. The first population derived from the cross "DH24/DH36" (Pop 1), being DH24 a line with high values of FFE, FE and FEm, whereas DH36 a line with low values of FFE, FE and FEm. The second population derived from the cross "DH24/DH22" (Pop 2), being DH24 a line with high FEm, as in the first population, and DH22 a line with low FEm. Before selecting the contrasting lines for crosses, they were considered for showing segregation for the *QFFE.perg-5A* (Pop 1) or *QFEm.perg-3A* (Pop 2) but were fixed for *Vrn-A1b* (Yan et al. 2004) and *Vrn-B1* (Fu et al. 2005) spring alleles. Furthermore, in the case of plant height, a stable QTL located on chromosome 6A (51.2 cM) was fixed, using the tall allele from B2002 (Mo et al. 2019). Finally, selected lines were checked for being monomorphic for the photoperiod-insensitive *Ppd-D1a* allele, using PCR markers developed by Beales et al. (2007). Prior to obtaining the F_2, the F_1 seeds from each cross were checked for its heterozygosity by analyzing the composition of high molecular weight glutenins (HMWG) by SDS PAGE according to Pflüger et al. (2001) using half grain without embryo.

Genotyping and genetic map construction

B19/B2002 DH population was genotyped with the iSelect 90K SNP assay (Wang et al. 2014). For genetic map construction, SNPs markers with more than 20% of missing and/or heterozygous data were discarded. The Python script, merger.py,[1] was used to group all SNPs that showed identical segregation in the population before the map construction. Finally, the R package "Rqtl" (Broman et al. 2003) was used for the genetic map development. Additionally,

[1] https://github.com/juancrescente/lmap.

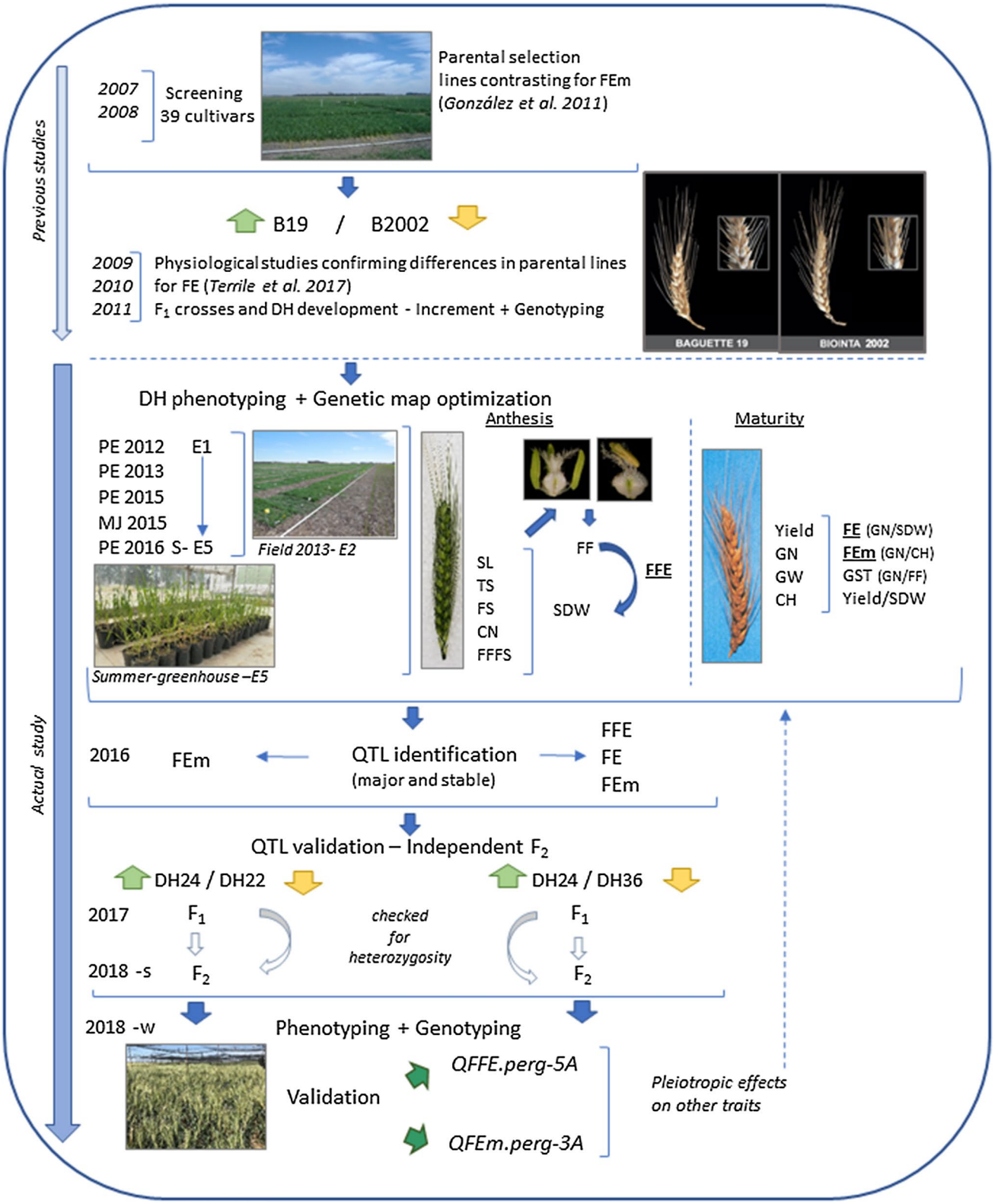

Fig. 1 Workflow showing the timeline, genetic populations, environments and measured variables. E1 to E5: environments for DH phenotyping. *PE* Pergamino, *MJ* Marcos Juarez, *S* summer, *W* winter, *FFE* fertile floret efficiency, *FE* fruiting efficiency, *FEm* fruiting efficiency at maturity, *SL* spike length, *TS* total spikelets per spike, *FS* fertile spikelets per spike, *CN* Compactness, *CH* Chaff—no-grain spike dry weight at maturity, *FF* fertile florets per spike, *FFFS* fertile florets per fertile spikelet, *SDW* spike dry weight at anthesis, *GST* grain set, *GN* grain number per spike, *GW* grain weight

Table 1 Environment description of the B19/B2002 DH population phenotyping

Environment	Location and year[a]	Blocks/ repeats	Experimental unit	Traits phenotyped
E1	PE2012	2	Plot	SDW, FF, FFE, SL, TS, FS, FFFS, CN, CH
E2	PE2013	2	Plot	SDW, FF, FFE, SL, TS, FS, FFFS, CN, GST, CH, FE, FEm, GN
E3	PE2015	2	Plot	SDW, FF, FFE, SL, TS, FS, FFFS, CN, GST, CH, FE, FEm, GN, GW
E4	MJ2015	2	Plot	CH, FEm, GN, GW
E5	PE2016	6	Pot	SDW, FF, FFE, SL, TS, FS, FFFS, CN, GST, CH, FE, FEm, GN, GW

SDW spike dry weight at anthesis, *FF* fertile florets per spike, *FFE* fertile floret efficiency, *SL* spike length, *TS* total spikelets per spike, *FS* fertile spikelets per spike, *FFFS* fertile florets per fertile spikelet, *CN* Compactness of the spike, *GST* grain set, CH Chaff (no-grain spike dry weight at maturity), *FE* fruiting efficiency, *FEm* fruiting efficiency at maturity, *GN* grain number per spike, *GW* grain weight

[a]All experiments were performed in the field except in E5. PE: Pergamino, Buenos Aires, Argentina; MJ: Marcos Juárez, Córdoba, Argentina

two functional markers for the vernalization genes *Vrn-A1* (Yan et al. 2004) and *Vrn-B1* (Fu et al. 2005) were added to the DH genetic map for this study. The physical position of the SNPs was determined by BLAST against the IWGSC Ref. Seq. v1.0 wheat genome assembly (Appels et al. 2018).

The F_2 populations for the QTL validations (Pop 1 and Pop 2) were genotyped using KASP (Kompetitive Allele Specific PCR, LGC-Genomics, UK) assay with primers designed using PolyMarker (Ramirez-Gonzalez et al. 2015) (Table S1, Online Resource 1). The KASP assays were run in a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) under the following thermal conditions: 95 °C for 15 min (hot start enzyme activation), 95 °C for 30 s, 65 °C for 1 min (touchdown over 65–57 °C for 60 s), ten cycles (dropping 0.8 °C per cycle), and 72 °C for 30 s (11 cycles); 94 °C for 30 s, 57 °C for 1 min and 72 °C for 30 s (26 cycles); and 72 °C for 5 min and 20 °C (final).

Phenotyping

Experiments performance and general conditions

The phenotyping for the QTL mapping of the DH population included five environments (Table 1, Fig. 1). The experiments in E1 to E4 were performed under field conditions within the optimum sowing date for the area (central region of wheat growing in Argentina). Plots consisted of (a) two rows 1 m long and 0.21 m apart in E1 (190 plants m^{-2}) and (b) five rows 2 m long and 0.21 m apart in E2–E4 (E2: 330 plants m^{-2}, and E3–E4: 280 plants m^{-2}). Field experiments were conducted in a randomized complete block design (RCBD) with two replications. A nonagronomic experiment was performed during the summer season in a greenhouse to explore a stressful environment (E5). The plants were transplanted during February after artificial vernalization in cool chamber (20 days at 5 °C, 8 h light). Greenhouse experiment was conducted in pots (five plants per pot of 5 l capacity, filled with soil from the area) in a RCD with six replications. Every 10 days, pots were re-arranged within the greenhouse to avoid border effects.

The experiments for the QTL validation using the F_2 populations were performed under field conditions, protected by an anti-bird net, at EEA Pergamino (33° 51′ S, 60° 56′ W) Research Station of INTA (National Institute of Agricultural Technology and Husbandry, Argentina). Seeds of both F_2 populations were sown on June 19 at 0.30 m each in rows 1.20 m long and 0.20 m apart. For all the experiments, pest and diseases were prevented by appropriate chemical application, and high fertilization and irrigation were performed.

Description of sampling and traits phenotyped

Plots of the DH population were sampled at the anthesis stage (Z6.1, Zadoks et al. 1974). In E1, five of the most representative spikes of each row were cut. In E2 and E3, a half meter of a central row was sampled, and the spikes were separated from the rest of the biomass. Then, the spikes were arranged by length and the three median spikes were selected. In E5, three main stem spikes (one of each pot) were cut. In E4, no measurement at anthesis was made. The spike length (SL, mm) was measured from the base of the first spikelet to the terminal spikelet using an electronic caliper. The number of total spikelets per spike was counted (TS) and the spike compactness (CN, mm spikelet $node^{-1}$) was estimated as the ratio between SL and TS. The number of fertile florets of each spike (FF) was counted using a binocular microscope. The floret was considered fertile when yellow anthers were visible, or the floret score was > 9.5 in Waddington et al. (1983) scale. The fertile florets were counted in a half side of the spike ("a") and in the terminal spikelet ("t"). The final number per spike was estimated multiplying "a" by 2 and adding the "t" value. The number of fertile spikelets (FS) was estimated as the number of spikelets with at least one fertile floret multiplied by 2 and adding the terminal spikelet in case it was fertile. The number of

fertile florets per fertile spikelet (FFFS) was estimated as the ratio between FF and FS. The spike dry weight (SDW) was determined after drying in an oven at 70 °C for 48 h. The FFE was estimated as the ratio between FF and SDW.

At maturity (Z9, Zadoks et al. 1974), a new sampling was performed in the DH population. In E2, E3 and E5, the spikes were selected following the same criteria as in anthesis. In E4, the spikes were selected following the same criteria as in E2 and E3. In E1, fusarium head blight was present despite the fungicide application; then, data from maturity in this environment are not available. All the spikes were dried in an oven at 70 °C for 48 h and weighed before threshing by hand. The grains were weighed before counting with an automatic counter to estimate the number of grains per spike (GN) and the average grain weight (GW). The CH (chaff—no-grain spike dry weight at maturity) was estimated by subtracting the weight of the grains from the dry weight of the spike at maturity. The FEm was estimated as the ratio between GN and CH, while the FE was estimated as the ratio between GN and SDW. The GST was estimated as the relation GN/FF.

The F_2-plants of Pop 1 were evaluated for fertile floret efficiency at anthesis (FFE), fruiting efficiency (FE) and fruiting efficiency at maturity (FEm), whereas the F_2-plants of the Pop 2 were evaluated for fruiting efficiency at maturity (FEm) (Fig. 1), which were estimated as described previously for the DH population. Sampling was adjusted to the one-plant design as experimental unit. Then, in Pop 1, the main stem of each individual plant was cut at anthesis and the first tiller was cut at maturity, while in Pop 2 main stem of each individual plant was cut at maturity. In Pop 2, no measurements at anthesis were performed. To estimate FFE, the number of FF of each spike was counted in each spikelet of the spike (instead of one side as in the DH).

QTL and statistical analysis

QTL analyses for the DH population were conducted using composite interval mapping (CIM) with forward and backward regressions and 500 permutations at $\alpha = 0.05$ as implemented in the publicly available software QTL Cartographer 2.5 (Wang et al. 2012). The QTL analysis was performed on data for each environment separately, after averaging the scores for each of the DH lines across all replications within each environment. Additionally, the Best Linear Unbiased Estimator (BLUE) was calculated for each DH line including all tested environments as random variable. The BLUE values were treated like an additional environment in the QTL mapping. A LOD value of 2.5 was selected as a uniform threshold for all analyses. QTL were considered stable if they were detected at least in three environments and if they were defined as major QTL (i.e., $R^2 > 10\%$ at least in one environment).

For each of the traits evaluated in the DH population, we performed a factorial ANOVA using the QTL peak marker as class variables in the model, together with all possible two-way interactions. Environments were included as blocks (a random class variable). This analysis was used to determine the potential epistatic interactions among loci. For the F_2 populations, one-way ANOVAs were conducted separately for each QTL. Data violating the ANOVA assumptions (normality of residuals by Shapiro–Wilk tests and homogeneity of variances by Levene's tests) were corrected using power transformations. All statistical analyses were conducted using Infostat/P (Di Rienzo et al. 2016).

The additive effect (a), dominance effect (d) and dominance degree ($D = d/a$) were estimated for the QTL of interest. The degree of dominance was calculated as:

$$D = \frac{2x_2 - x_1 - x_3}{x_1 - x_3}$$

where x_1, x_2 and x_3 are the phenotypic values of the homozygote with the increased value, the heterozygote value and the other homozygote with the decreased value (Falconer 1960; Stone 1968). $D = 0$ would be indicative of nondominance, $D = 1$ of complete dominance, $0 < D < 1$ of incomplete dominance, $-1 < D < 0$ of incomplete recessive and $D = -1$ of complete recessive (Stone 1968).

Results

Phenotypic performance of the DH population

The FFE, FE and FEm showed a normal distribution for all environments, ranging from 94 ± 12 to 149 ± 35 florets g_{SDW}^{-1}, 105 ± 39 to 145 ± 32 grains g_{SDW}^{-1} and 70 ± 24 to 136 ± 19 grains g_{CH}^{-1}, respectively. Transgressive segregation was observed with greater and lower values than the parental lines (Table 2, Fig. S1, Online Resource 2).

The variation in FFE, FE and FEm was highly explained by the environment, and the genotype but GxE was significant in FFE and FEm. The narrow-sense heritability was 0.52 for FFE, 0.61 for FE and 0.63 for FEm (Table S2, Online Resource 1). Detailed results about narrow-sense heritability and relationship among traits are presented in Pretini et al. (2020).

QTL mapping for FFE, FE and FEm in B19/B2002 DH population

The final linkage map consisted of 10,936 SNPs distributed in 739 loci across the 21 wheat chromosomes, giving a total map length of 2,270.8 cM, with an average locus spacing of 3.2 cM (Table S3, Online Resource 1). A total of

Table 2 Population distribution and parental means for each environment (E1–E5)

Trait	B19/B2002							B19		B2002	
	Environment	Mean	SD	*n*	*W*[a]	*P* value	*r*[b]	Mean	SEM	Mean	SEM
FFE	E1	94	11.9	95	0.99	0.9703		103	3.30	84	0.30
	E2	143	16.9	87	0.98	0.5582		152	1.60	135	8.50
	E3	137	20.1	100	0.98	0.8059		149	3.40	113	0.00
	E5	149	34.9	102	0.97	0.1769		146	6.20	137	20.80
FE	E2	124	25.4	87	0.96	0.1080		124	1.95	104	1.80
	E3	145	32.3	99	0.96	0.0337	0.98	154	23.30	122	4.20
	E5	105	39.1	101	0.98	0.4816		136	20.50	93	14.10
FEm	E2	136	19.1	107	0.97	0.2075		148	7.10	111	0.35
	E3	86	12.9	105	0.97	0.1198		100	3.80	72	0.40
	E4	89	13.1	103	0.95	0.0060	0.96	106	3.20	80	0.65
	E5	70	23.8	101	0.97	0.2354		66	0.30	49	0.88

SD standard error, *n* number of lines phenotyped, *SEM* standard error of the mean. Edited from Pretini et al. (2020)

[a] *W* = Shapiro–Wilk edited by Mahibbur and Govindarajulu (1997)

[b] Q–Q plot normal distribution

37 QTL were detected across all environments and BLUE analysis. The QTL were distributed on 14 of the 21 wheat chromosomes (Table 3). The allele for high FFE, FE and FEm was contributed by B19 in 34 of the 37 QTL detected. The B2002 cultivar contributed to the three remaining QTL, two of them associated with FFE and the third one with FE. The peak marker of the two QTL for FFE was located on the 5B chromosome coincident with the vernalization response gene *Vrn-B1* (the spring allele from B2002 increased the FFE). However, this 5B QTL was detected at the greenhouse environment which was conducted during summer indicating that it could be a phenology effect on the character. The remaining QTL contributed by B2002 and detected for FE was located on the 7B chromosome, but it was present only in one environment and the peak marker explained only 5% of the observed variance so it was not considered as significant. Only one of the QTL contributed by B19 was associated with the already known gene *Vrn-A1*. It was also detected in the summer experiment on chromosome 5A (the spring allele from B19 increased the FFE). The rest of the QTL contributed by B19 were novel and are described in the following items.

QTL for FFE

The QTL analysis identified six regions on chromosomes 1A, 2A, 5A, 5B and 6B for FFE (Table 3), but only two of them were stable across environments. The 5A QTL was consistent in three of four tested environments and also in the BLUE. The peak of this QTL was mapped at the BS00083507_51 SNP marker (42.1 cM, 461.49 Mb) with a maximum LOD of 5.05 (Fig. 2a, Fig. S3a, Online Resource 2). In the factorial ANOVA, BS00083507_51 explained 11.0–23.0% of the observed variation in FFE. The FFE average difference between BS00083507_51 B19 and B2002 alleles was 8.4–19.2 florets g_{SDW}^{-1}. The 1A QTL which was consistent across two of four tested environments and in the BLUE values was mapped at the RAC875_c54245_88 SNP marker (74.4 cM, 15.43 Mb) with a maximum LOD of 5.00 (Fig. 2b, Fig. S3b, Online Resource 2). In the factorial ANOVA, RAC875_c54245_88 explained 18.0–21.0% of the observed variation in FFE. The FFE average difference between RAC875_c54245_8 B19 and B2002 alleles was 18.2–21.9 florets g_{SDW}^{-1} (Table 3).

QTL for FE

For the FE, the QTL analysis identified 7 regions on chromosomes 1B, 1D, 2D, 4D, 5A, 6B and 7B (Table 3). The most stable was the 5A QTL which was consistent in 2 of 3 tested environments and in the BLUE values. The peak of this QTL, as for FFE, was mapped at the BS00083507_51 SNP marker (42.1 cM, 461.49 Mb) with a maximum LOD of 6.46 (Fig. 2c, Fig. S3c, Online Resource 2). In the factorial ANOVA, BS00083507_51 explained 11.0–26.0% of the observed variation in FE. The FE average difference between BS00083507_51 B19 and B2002 alleles was 22.4–29.4 grains g_{SDW}^{-1} (Table 3).

QTL for FEm

Eight regions on chromosomes 1A, 2D, 3A, 4D, 5A, 6A and 7A were identified for FEm (Table 3). The 1A and the 2D QTL were mapped in the same positions as the ones observed for FFE and FE, respectively, but both were only present in one environment (E3). In contrast,

Table 3 QTL identified for FFE, FE and FEm in the five tested environments

Trait	Chr.	Env.	Peak marker[a]	Genetic (cM)	Physical (Mb)	LOD	Donor	Add	R^2 (%)
FFE	1A	E2	RAC875_c54245_88	74.4	15.4	4.36	B19	18.6	21
	1A	E3	RAC875_c54245_88	74.4	15.4	5.00	B19	21.9	19
	1A	BLUE	RAC875_c54245_88	74.4	15.4	4.36	B19	18.2	18
	2A	E1	BS00065434_51	106.2	774.1	4.55	B19	9.6	14
	5A	E1	BS00083507_51	42.1	461.5	3.98	B19	8.4	11
	5A	E2	BS00083507_51	42.1	461.5	5.05	B19	11.1	12
	5A	E3	BS00083507_51	42.1	461.5	3.54	B19	19.2	23
	5A	BLUE	BS00083507_51	42.1	461.5	4.12	B19	15.0	20
	5A	E5	*Vrn-A1*	75.3	587.4	7.18	B19	24.3	13
	5B	E5	*Vrn-B1*	64.0	573.8	8.23	B2002	31.0	21
	5B	BLUE	*Vrn-B1*	64.0	573.8	2.90	B2002	12.2	13
	6B	E3	Ex_c17379_1431	66.6	692.8	2.82	B19	13.2	10
FE	1B	E2	Ra_c21994_996	62.5	656.8	3.24	B19	17.8	12
	1D	E5	Excalibur_c15692_532	36.7	12.1	2.82	B19	22.5	10
	1D	BLUE	Excalibur_c15692_532	36.7	12.1	2.50	B19	14.7	8
	2D	E3	BS00079440_51	82.3	79.4	6.01	B19	31.0	21
	2D	BLUE	BS00079440_51	82.3	79.4	5.04	B19	22.2	18
	4D	E5	Kukri_rep_c68594_530	24.2	12.7	2.65	B19	21.5	9
	5A	E3	BS00083507_51	42.1	461.5	4.09	B19	22.4	11
	5A	E5	BS00083507_51	42.1	461.5	2.91	B19	29.4	17
	5A	BLUE	BS00083507_51	42.1	461.5	6.46	B19	26.7	26
	6B	E2	BS00063109_51	40.3	57.8	4.38	B19	12.9	7
	7B	E3	RAC875_c27939_335	34.4	349.8	2.67	B2002	15.1	5
FEm	1A	E3	RAC875_c54245_88	74.4	14.4	4.20	B19	14.2	19
	1A	E4	RAC875_c53185_802	149.3	480.5	4.57	B19	9.9	11
	2D	E3	BS00079440_51	82.3	79.4	3.74	B19	13.1	25
	3A	E2	wsnp_CAP11_rep_c4226_1995152	51.6	685.1	3.63	B19	13.2	11
	3A	E4	wsnp_CAP11_rep_c4226_1995152	51.6	685.1	4.39	B19	10.1	14
	3A	E5	wsnp_CAP11_rep_c4226_1995152	51.6	685.1	3.69	B19	13.6	9
	3A	BLUE	wsnp_CAP11_rep_c4226_1995152	51.6	685.1	4.00	B19	10.2	14
	4D	BLUE	BS00099053_51	2.9	3.6	2.53	B19	7.1	7
	5A	E3	BS00083507_51	42.1	461.5	3.04	B19	9.4	13
	5A	E5	BS00083507_51	42.1	461.5	2.50	B19	22.1	23
	5A	BLUE	BS00083507_51	42.1	461.5	4.93	B19	11.5	19
	6A	E2	BS00082191_51	44.3	7.6	2.69	B19	17.0	18
	7A	E4	wsnp_Ku_rep_c113718_96236830	68.3	625.7	3.62	B19	7.1	7

Chr. chromosome, *Env.* environment, *Add* additive effect

[a]Closest marker to the highest LOD score

the 5A QTL was consistent in two of four tested environments and in the BLUE. The peak of this QTL was also mapped at the BS00083507_51 SNP marker (42.1 cM, 461.49 Mb), similar to the ones identified for FFE and FE, with a maximum LOD of 4.93 (Fig. 2d, Fig. S3d, Online Resource 2). In the factorial ANOVA, BS00083507_51 explained 13.0–23.0% of the observed variation in FEm. The average difference between BS00083507_51 B19 and B2002 alleles was 9.4–22.1 grains g_{CH}^{-1} (Table 3). Another promising QTL was the one present on the 3A chromosome, which was consistent across three of four tested environments. The peak QTL mapped at the wsnp_CAP11_rep_c4226_1995152 SNP marker (51.6 cM, 685.12 Mb) with a maximum LOD of 4.00 (Fig. 2e, Fig. S3e, Online Resource 2). In the factorial ANOVA, wsnp_CAP11_rep_c4226_1995152 explained 9.0–14.0% of the observed variation in FEm (Table 3). The average difference between wsnp_CAP11_rep_c4226_1995152 B19 and B2002 alleles was 10.1–13.6 grains g_{CH}^{-1} (Table 3).

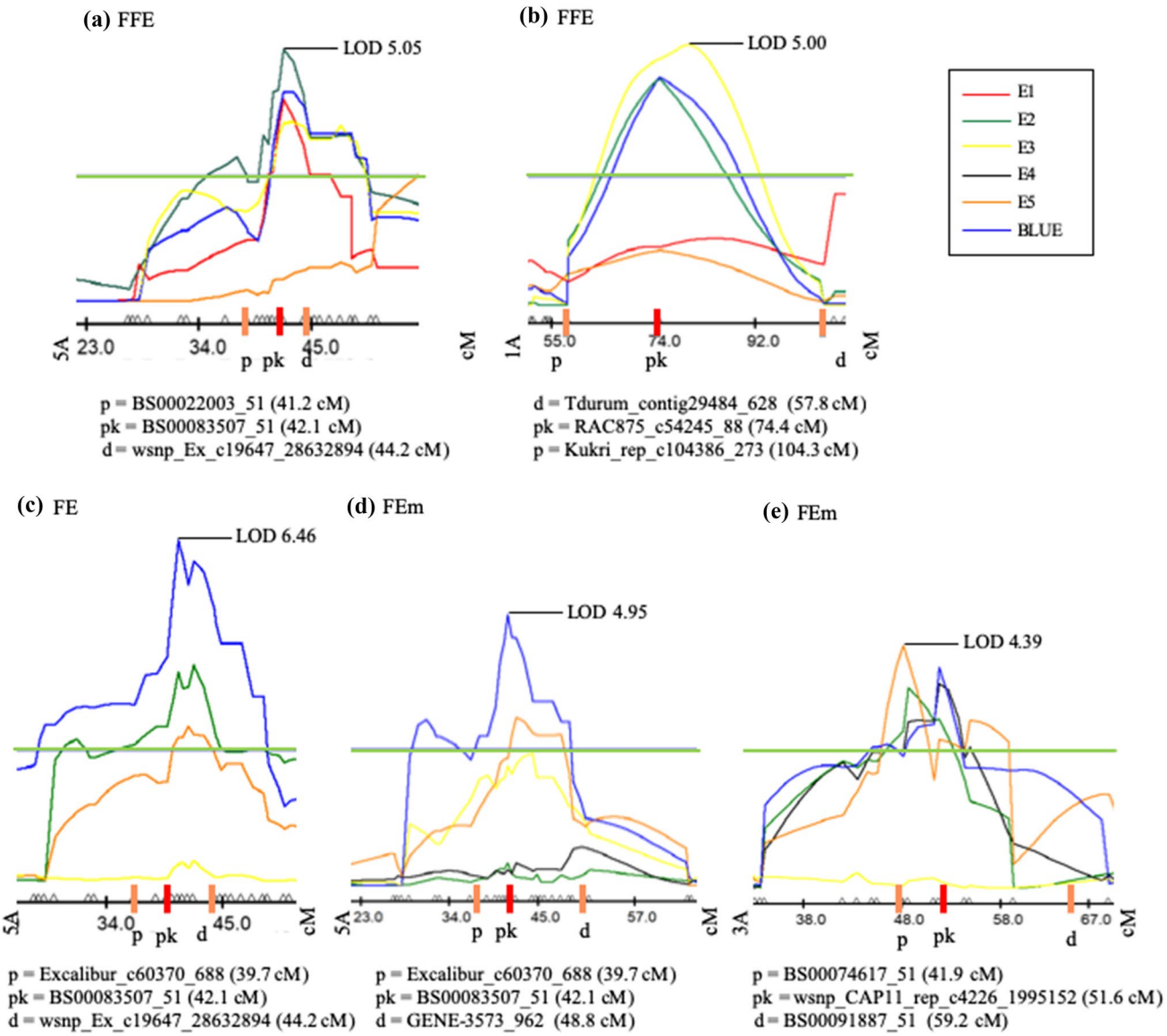

Fig. 2 Main QTL detected in the B19/B2002 DH mapping population for **a** FFE on chromosome 5A, **b** FFE on chromosome 1A, **c** FE on chromosome 5A, **d** FEm on chromosome 5A and **e** FEm on chromosome 3A. The highest peak LOD scores are indicated, along with the light green horizontal line indicating the threshold LOD (2.5). Lines of different colors indicate different environments for each trait. *pk* peak marker (red tick marks), *d* distal flanking marker and *p* proximal flanking marker (orange tick marks) (color figure online)

Consolidated QTL

Considering as stable those QTL present in at least three environments with a LOD > 2.5 and as major QTL if they present a $R^2 > 10\%$ in at least one environment, we detected two major and stable QTL. The first was the one identified at the SNP BS00083507_51 located on the chromosome 5A (42.1 cM, 461.49 Mb), henceforth *QFFE.perg-5A*. It was detected in the largest number of environments in the QTL analysis for FFE and also observed in three and two environments for FE and FEm (including BLUE values). The second was the one identified with the SNP wsnp_CAP11_rep_c4226_1995152 located on the chromosome 3A (51.6 cM, 685.12 Mb), henceforth *QFEm.perg-3A*. It was detected in the largest number of environments in the QTL analysis for FEm.

Considering a generalized linear mixed model with the environments as random effects, the effect of the *QFFE.perg-5A* and *QFEm.perg-3A* on FFE was significant ($P < 0.0001$ and $P = 0.048$, respectively) while no epistatic interactions were observed ($P = 0.6199$). Plants carrying the *QFFE.perg-5A* and *QFEm.perg-3A* allele for high FFE (B19) had 15.7 and 4.1 more fertile florets established per unit of spike dry weight at anthesis than those carrying the

low FFE allele (B2002), respectively (Table 4). The effect of the *QFFE.perg-5A* and *QFEm.perg-3A* on FE was significant ($P < 0.0001$ and $P = 0.004$, respectively), while no epistatic interactions was observed ($P = 0.5616$). Plants carrying the *QFFE.perg-5A* and *QFEm.perg-3A* alleles for high FE (B19) had 27.0 and 11.8 more grains per unit of spike dry weight at anthesis than those carrying the low FE allele (B2002), respectively (Table 4). Finally, the effect of the *QFFE.perg-5A* and *QFEm.perg-3A* on FEm was highly significant (both $P < 0.0001$), while no epistatic interaction was observed ($P = 0.8162$). Plants carrying the *QFFE.perg-5A* and *QFEm.perg-3A* allele for high FEm (B19) had 13.0 and 9.5 more grains per unit of CH than those carrying the low FEm allele (B2002), respectively (Table 4).

Phenotypic performance of the F_2 populations

For validating the two identified QTL, two F_2 populations were developed, Pop 1 for the *QFFE.perg-5A* and Pop 2 for the*QFEm.perg-3A* (see "Plant materials and populations development" section). The mean anthesis date of both F_2 populations took place during October and was very close among lines. The F_2 populations consisted of 434 and 490 plants each, and the traits phenotyped showed a normal distribution. In Pop 1, the mean of the three traits FFE, FE and FEm was 135 ± 15 florets g_{SDW}^{-1}, 128 ± 23 grains g_{SDW}^{-1} and 95 ± 15 grains g_{CH}^{-1}, respectively (Fig. S2a, b and c, Online Resource 2). Meanwhile, in Pop 2, the mean FEm was 92 ± 13 grains g_{CH}^{-1} (Fig. S2d, Online Resource 2).

From the ca. 430–500 phenotyped plants, only 264 plants from Pop 1 and 220 plants from Pop 2 were necessary for the QTL validation. As well as the complete populations, both subpopulations showed a normal distribution and covered all the phenotypic variance. In Pop 1, the FFE ranged from 104 to 172 florets g_{SDW}^{-1} with a mean of 132 ± 12 florets g_{SDW}^{-1}, the FE from 72 to 189 grains g_{SDW}^{-1} with a mean of 124 ± 20 grains g_{SDW}^{-1} and the FEm from 58 to 136 grains g_{CH}^{-1} with a mean of 93 ± 12 grains g_{CH}^{-1} (Fig S2e, f and g, Online Resource 2). In the Pop 2, the FEm ranged from 62 to 122 grains g_{CH}^{-1} with a mean of 92 ± 11 grains g_{CH}^{-1} (Fig. S2h, Online Resource 2).

QTL *QFFE.perg-5A* and *QFEm.perg-3A* validation

To validate the position and effect of the *QFFE.perg-5A*, the independent F_2 population (Pop 1) was genotyped using the *QFFE.perg-5A* peak SNP (BS00083507_51) transformed to KASP marker (Table S1, Online Resource 1). As we mentioned before (see "Plant materials and populations development" section), Pop 1 was developed in a homogeneous genetic background for the main segregating adaptation genes (*Vrn-A1, Vrn-B1)* and fixed *QFEm.perg-3A* B19 allele in order to evaluate the effect of the 5A QTL only. From the 264 plants of Pop 1 used for validation of *QFFE.perg-5A*, 68 were found to be homozygous for the B19 allele, 75 were homozygous for the B2002 allele and the remaining 121 plants were found to be heterozygous for the *QFFE.perg-5A* peak KASP marker. For FFE, the one-way ANOVA revealed a significant effect of the *QFFE.perg-5A* peak marker on the trait ($P = 0.0003$, Fig. 3a). A similar effect was detected for FE ($P = 0.0009$, Fig. 3b) and FEm ($P = 0.0004$, Fig. 3c). In all cases, the heterozygous plants showed less florets or grains than expected by a pure additive effect (FFE = −1.35 florets g_{SDW}^{-1}, FE = −2.62 grains g_{SDW}^{-1} and FEm = −2.52 grains g_{CH}^{-1}, respectively). The degree of dominance (*D*) (Falconer 1960) of *QFFE.perg-5A* B19 allele for increasing the FFE, FE and FEm was −0.34, −0.44 and −0.68, respectively, indicative in all cases of incomplete recessive.

To validate the position and effect of the *QFEm.perg-3A*, the independent F_2 population (Pop 2) was genotyped using the *QFEm.perg-3A* peak SNP (wsnp_CAP11_rep_c4226_1995152) transformed to KASP marker (Table S1, Online Resource 1). Similar to Pop 1, the Pop 2 population was developed in a homogeneous background in order to minimize the effect of phenology genes (see "Plant materials and populations development" section). Additionally, the B19 allele for *QFFE.perg-5A* was fixed in the segregating population in order to evaluate the effect of the 3A QTL only.

Of the 220 plants, 50 were found to be homozygous for the high FEm B19 allele, 39 were found to be homozygous for the low FEm B2002 allele and the remaining 131 plants were found to be heterozygous for the *QFEm.perg-3A* peak KASP marker. One-way ANOVA revealed a significant effect of the *QFEm.perg-3A* KASP marker on FEm ($P = 0.0033$, Fig. 3d). The heterozygous plants showed 0.35 grains g_{CH}^{-1} more than expected by a pure additive effect, and the degree of dominance (*D*) of B19 allele for increased FEm was roughly 0.09 indicative of incomplete dominance.

Pleiotropic effects and interactions between *QFFE.perg-5A* and *QFEm.perg-3A* on other traits of the DH population

We also analyzed the effects of *QFFE.perg-5A* and *QFEm.perg-3A* and their two-way interactions on other traits of the DH population related to FF and GN determination (Table 4, Fig. 4).

The positive effect of *QFFE.perg-5A* B19 allele on FFE (+11%) resulted in a 4% increase in the FF despite the 6% reduction of SDW (Table 4, Fig. 4). The GN was also improved by this allele by 9%, not only due to the effect on FF but also by the increment of GST by 13%. Considering the spike structure traits, the lines with the *QFFE.perg-5A*

Table 4 Effects of *QFFE.perg-5A* and *QFEm.perg-3A* on different traits

		Main effect		Two-way interaction
Trait	Value	*BS00083507_51* (5A)	*wsnp_CAP11_rep_c4226_1995152* (3A)	5A×3A
FFE	B19 allele	139.4	133.8	
$N^a = 557$	B2002 allele	123.7	129.7	
Difference (%)		11.3%	3.1%	
$Env^b = 4$	*P*	****	*	ns
FE	B19 allele	137.8	130.2	
$N = 409$	B2002 allele	110.8	118.4	
Difference (%)		19.6%	9.1%	
Env = 3	*P*	****	**	ns
FEm	B19 allele	100.6	98.9	
$N = 569$	B2002 allele	87.2	89.3	
Difference (%)		13.3%	9.7%	
Env = 3	*P*	****	****	ns
FF	B19 allele	45.1	44.6	
$N = 561$	B2002 allele	43.4	43.9	
Difference (%)		3.8%	1.6%	
Env = 4	*P*	**	ns	ns
GST	B19 allele	0.93	0.90	
$N = 408$	B2002 allele	0.81	0.84	
Difference (%)		12.9%	6.7%	
Env = 3	*P*	****	*	ns
GN	B19 allele	40.1	39.3	
$N = 580$	B2002 allele	36.5	37.3	
Difference (%)		9.0%	5.1%	
Env = 4	*P*	****	**	ns
SL	B19 allele	90.8	92.9	
$N = 547$	B2002 allele	97.2	95.1	
Difference (%)		−7.0%	−2.4%	
Env = 4	*P*	****	**	*
TS	B19 allele	20.7	20.8	
$N = 562$	B2002 allele	21.4	21.3	
Difference (%)		−3.4%	−2.4%	
Env = 4	*P*	****	**	ns
FS	B19 allele	17.1	16.9	
$N = 563$	B2002 allele	17.0	17.2	
Difference (%)		0.6%	−1.8%	
Env = 4	*P*	ns	*	ns
FFFS	B19 allele	2.61	2.60	
$N = 563$	B2002 allele	2.51	2.51	
Difference (%)		3.8%	3.5%	
Env = 4	*P*	****	***	ns
CN	B19 allele	4.43	4.50	
$N = 547$	B2002 allele	4.56	4.49	
Difference (%)		−2.9%	0.2%	
Env = 4	*P*	***	ns	**
SDW	B19 allele	0.361	0.367	
$N = 564$	B2002 allele	0.384	0.378	
Difference (%)		−6.4%	−3.0%	
Env = 4	*P*	***	ns	ns

Table 4 (continued)

		Main effect		Two-way interaction
Trait	Value	*BS00083507_51* (5A)	*wsnp_CAP11_rep_c4226_1995152* (3A)	5A×3A
CH $N=727$	B19 allele	0.422	0.422	
	B2002 allele	0.444	0.443	
Difference (%)		−5.2%	−5.0%	
Env = 5	*P*	**	**	ns
GW $N=447$	B19 allele	35.1	35.2	
	B2002 allele	36.9	36.8	
Difference (%)		−5.1%	−4.5%	
Env = 3	*P*	***	**	***
Yield/SDW $N=301$	B19 allele	4.88	4.74	
	B2002 allele	4.12	4.26	
Difference (%)		15.7%	10.1%	
Env = 2	*P*	****	**	ns
Yield/spike $N=441$	B19 allele	1.36	1.35	
	B2002 allele	1.29	1.30	
Difference (%)		5.3%	3.4%	
Env = 3	*P*	*	ns	ns

The mean average values of each trait when the lines present one of the alleles (B19 or B2002) for *QFFE.perg-5A* and *QFEm.perg-3A* are presented, as well as the percent difference between the mean values within QTL alleles. The statistical significance of the main effect of each QTL (or peak marker) and interaction between them is indicated

P values are from two-way mixed-model ANOVAs with environment as a random variable and the two loci as fixed variables (*$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$; *ns* no significant differences)

FFE fertile floret efficiency (florets g_{SDW}^{-1}), *FE* fruiting efficiency (grains g_{SDW}^{-1}), *FEm* fruiting efficiency at maturity (grains g_{CH}^{-1}), *FF* fertile florets per spike (n° $spike^{-1}$), *GST* grain set (n° grains $floret^{-1}$), *GN* grain number per spike (n° $spike^{-1}$), *SL* spike length (mm), *TS* total spikelets per spike (n° $spike^{-1}$), *FS* fertile spikelets per spike (n° $spike^{-1}$), *FFFS* fertile florets per fertile spikelet (n° $spikelet^{-1}$), *CN* Compactness of the spike (mm $node^{-1}$), *SDW* spike dry weight at anthesis (g $spike^{-1}$), *CH* Chaff (no-grain spike dry weight at maturity, g $spike^{-1}$), *GW* grain weight (g), *Yield/SDW* yield per spike dry weight at anthesis (g $gSDW^{-1}$), *Yield/spike* yield per spike (g $spike^{-1}$)

[a]Number of experimental units measured. The number of blocks per environment is listed in Table 1

[b]Number of environments where the trait was evaluated

from B19 had reduced SL and CPMNS, resulting in less TS. However, the FS were not affected and the FFFS was increased (+4%), thus increasing the FF (Table 4, Fig. 4). Although the GW was reduced (−5%) due to the presence of the allele from B19, the efficiency to set yield per unit of spike dry weight at anthesis increased 16% (yield/SDW), resulting in a +5% yield per spike (Table 4, Fig. 4).

The *QFEm.perg-3A* allele from B19 showed no effect on FF or SDW, despite a slight increase in FFE (+3%) (Table 4, Fig. 4). Its positive effect on FEm (+10%) and FE (+9%) resulted in higher GN (+5%), though the improvement was lower than that of *QFFE.perg-5A*. This higher GN was consequence of increased GST by 7%, because as mentioned before the FF was similar between B19 and B2002 alleles. Similar to the *QFFE.perg-5A,* the presence of the B19 *QFEm.perg-3A* allele resulted in shorter spikes, with reduced TS, but in contrast to previous QTL, the FS was also reduced, and CN was not modified. Because of FS reduction, an increase in the FFFS was observed, but it resulted in similar FF due to the reduction in FS (Table 4, Fig. 4). Similar to *QFFE.perg-5A*, the presence of the *QFEm.perg-3A* allele from B19 reduced GW (−5%) and increased yield/SDW (+10%), but the effect on the yield per spike showed only a trend to increase (+3%).

Based on the two-way interaction between *QFEm.perg-3A***QFFE.perg-5A*, significant epistatic effects were observed for SL, CN and GW. In the case of SL, a significant epistatic interaction ($P=0.013$) was detected (Table 4). The *QFEm.perg-3A* B2002 allele increased significantly more the SL in the presence of the B2002 allele than in the presence of the B19 allele for the *QFFE.perg-5A* (Fig. S4a, Online Resource 2). For CN, the *QFEm.perg-3A* B2002 allele significantly reduced the CN under the presence of the B19 (high FE) allele for *QFFE.perg-5A* and significantly increased the CN under the presence of B2002 (low FE) allele for *QFFE.perg-5A* ($P=0.002$, Fig. S4b, Online

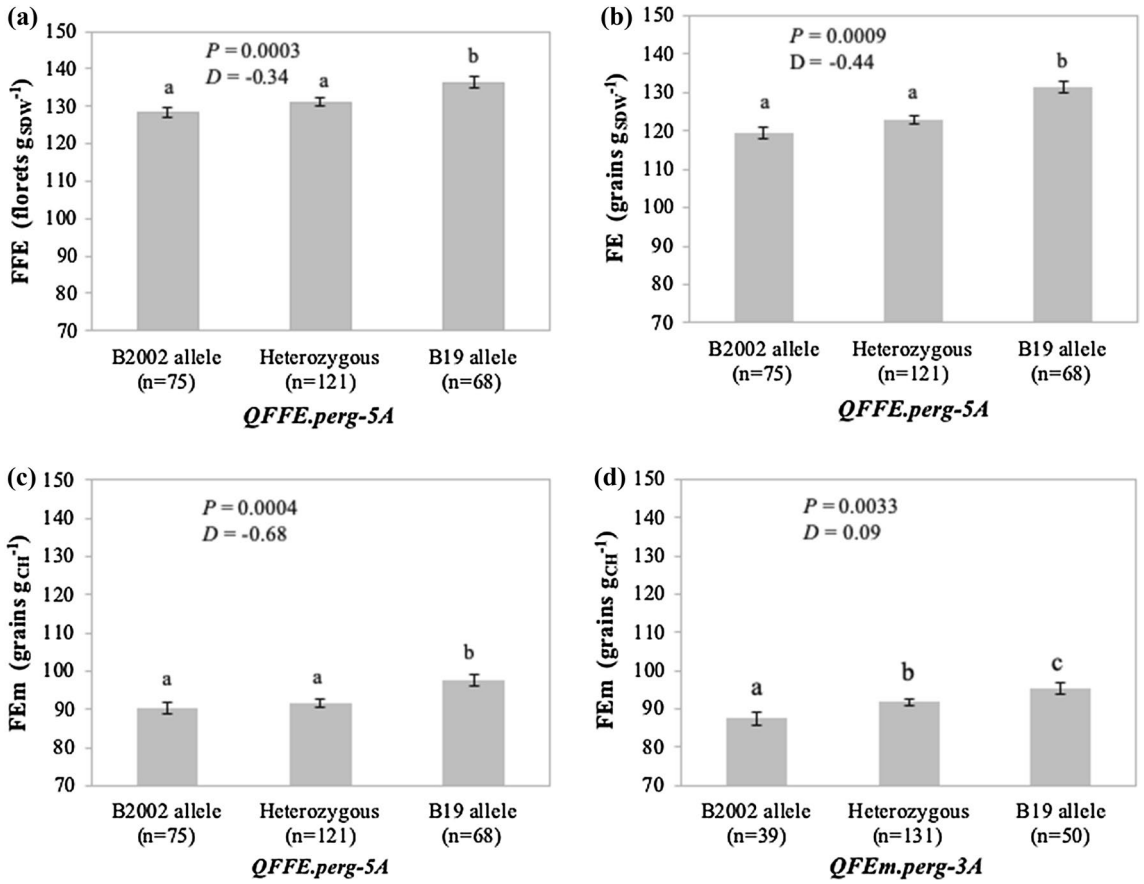

Fig. 3 Effects and degree of dominance (*D*) of the *QFFE.perg-5A* on **a** FFE, **b** FE, **c** FEm, evaluated from 264 F_2 plants and **d** effects and degree of dominance of the *QFEm.perg-3A* on FEm, evaluated from 220 F_2 plants. Different letters indicate significant differences between groups ($P < 0.05$)

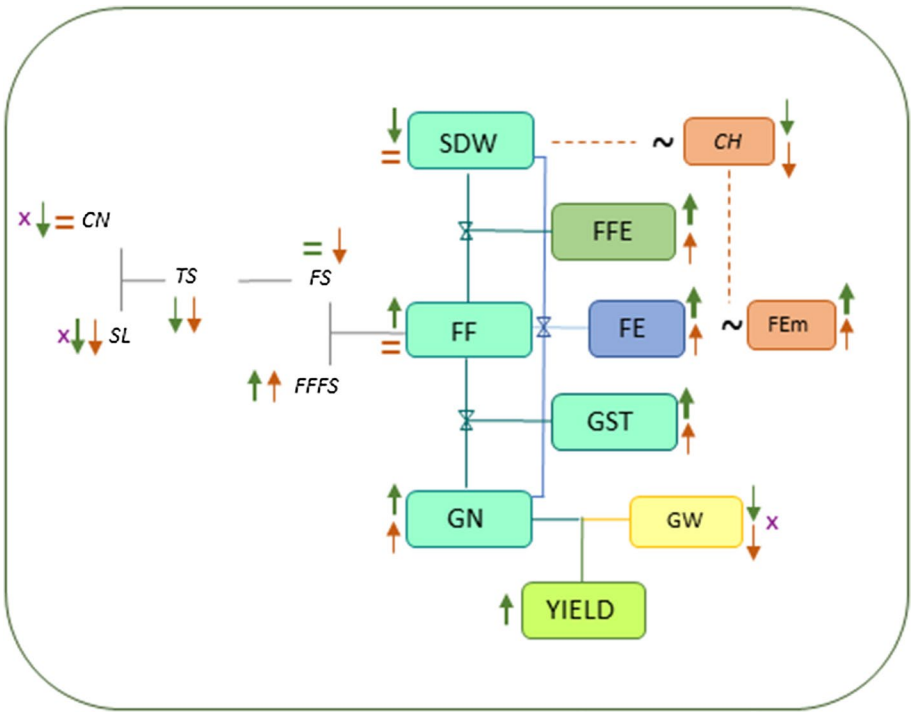

Fig. 4 Physiological conceptual framework of analysis of measured variables showing the main and pleiotropic effects of *QFFE.perg-5A* and *QFEm.perg-3A*. The symbols =, ↑, ↓, X indicate no effect, increment or reduction of the trait, and interaction between alleles, respectively, while the width of arrows indicates the magnitude of the effect. The green arrows represent *QFFE.perg-5A*, while the brown arrows represent *QFEm.perg-3A*. *FFE* fertile floret efficiency, *FE* fruiting efficiency, *FEm* fruiting efficiency at maturity, *SL* spike length, *TS* total spikelets per spike, *FS* fertile spikelets per spike, *FFFS* fertile florets per fertile spikelets, *CN* compactness, *FF* fertile florets per spike, *SDW* spike dry weight at anthesis, *GST* grain set, *GN* grain number, *GW* grain weight (color figure online)

Resource 2). Finally, for GW, the *QFEm.perg-3A* B2002 allele (low FE) increased significantly the GW only in the presence of the same parent (B2002) allele in the *QFFE.perg-5A* (P = 0.0004, Fig. S4c, Online Resource 2).

Discussion

The FE is a promising trait to improve grain number (and yield potential) in wheat, but it is difficult to measure, mainly because its sampling is destructive at anthesis to obtain the SDW, and impossible to use in early generations (Fischer and Rebetzke 2018). Then, it has been usually estimated at maturity, using the CH as a surrogate to SDW (FEm) (e.g., Stapper and Fischer 1990; González et al. 2011b; Martino et al. 2015; Mirabella et al. 2016; Alonso et al. 2018). However, as the relationship between chaff and SDW is unstable, the use of FEm may yield a wrong estimation of FE (Elía et al. 2016; Pretini et al. 2020). Therefore, the correlation between GN and FEm may be worse than that between GN and FE (Elía et al. 2016; Pretini et al. 2020), resulting in a reduced GN gain when selecting for FEm instead of FE (Pretini et al. 2020). To search for genetic bases, in the present work, we used a novel physiological approach based on Fischer (2011), where FE is the result of the efficiency of the spike to set fertile florets (FFE) and GST. As far as we know, there are no previous reports studying the genetic bases of FFE. In the present study, we identified several QTL associated to FFE, FE and FEm indicating that these traits are controlled by a complex genetic system. Nevertheless, two of them, *QFFE.perg-5A* and *QFEm.perg-3A*, were shown to be stable and major and validated in independent F_2-populations.

Previous studies (Guo et al. 2017; Gerard et al. 2019; Basile et al. 2019) focused only in FEm using GWAS populations. Guo et al. (2017) identified a genomic region on the 2A chromosome (676.2 Mb) associated with FEm, which was later identified at the *GNI-A1* gene (Sakuma et al. 2019). We detected only one region on the 2A chromosome (774.1 Mb) that was not considered as significant because it was present in only one environment (Table 3). However, the SNP positions of both works were 97.9 Mb apart, which would rule out the *GNI-A1* gene as candidate for our 2A QTL for FFE. On the other hand, Gerard et al. (2019) detected four SNPs on chromosomes 2A, 2D, 4D and 5A associated with FEm. The chromosome 5A-associated SNP was located at 698.5 Mb, 186 Mb distal from our *QFFE.perg-5A* QTL interval, suggesting it is not the same QTL.

Finally, Basile et al. (2019) working with a small Argentinean association mapping panel detected 17 genome regions (haplotypes) associated with FEm distributed on seven wheat chromosomes (1A, 2A, 3B, 4A, 5A, 6A and 7A). Particularly on chromosome 5A, they detected two associated regions; one of them comprises a small segment (14 Kb) at 445.2 Mb and another of 0.3 Mb between 476.4–476.7 Mb. Our *QFFE.perg-5A* QTL is located in the 391.4–512.2 Mb interval (± 1 LOD from peak), including the two regions detected by Basile et al. (2019). Interestingly, the parent cultivar carrying the high FE alleles used in our QTL mapping study (Baguette 19) was also included in the GWAS panel used by Basile et al. (2019) and showed the two haplotypes on chromosome 5A associated with high FE in the GWAS experiment. This information contributes further to the validation of the presence of a stable QTL for FEm on chromosome 5A that is found at least in moderate frequency in the Argentine wheat germplasm.

In the present work, based on a strong physiological model, we showed that the impact of this 5A QTL (*QFFE.perg-5A)* on the FEm is in fact consequence of its effect on FFE, as the QTL was more stable and major for this trait. Because of its association to FFE, the *QFFE.perg-5A* exhibited pleiotropic effects on associated traits, increasing the FF, the GST and then GN (Fig. 4). The fertile florets depend on the partitioning of assimilates to developing floret primordia (Ghiglione et al. 2008; González et al. 2011b), while the GST generally depends on the growth during post-anthesis (Fischer 1975, 1985), which was particularly true for the DH population used in this work (Pretini et al. 2020). As FF and GST increased and SDW and CH decreased when the *QFFE.perg-5A* B19 allele was present, its effect may be associated with an increased partitioning of dry matter to the developing florets/grains reducing partitioning to spike structure. This reduced partitioning to spike structure was accompanied by shorter spikes (-SL), and lower total spikelets (-TS) and internode length between spikelets (-CN), increasing the FS and FFFS. The action of this allele seems to be very different to the *GNI-A1* gene, which only increased the number of fertile florets per spikelet without any other change in the spike structure (Sakuma et al. 2019). The reduced GW observed when the *QFFE.perg-5A* from B19 was present confirms the negative association previously reported between FE and GW (Slafer et al. 2015; Terrile et al. 2017). This relation may be consequence in the present work of higher number of florets set in distal positions, which naturally have less carpel weight and potential grain weight (Calderini et al. 2001). We consider this nonconstitutive relation (Slafer et al. 2015) as the most probable because the higher FF resulted not only in higher efficiency to set yield per unit of spike weight at anthesis, but also in higher actual yield.

None of the previous reports (Guo et al. 2017; Gerard et al. 2019; Basile et al. 2019) detected a QTL associated with FEm in the chromosome 3A as we did (*QFEm.perg-3A*). As the FF was not statically improved, it seemed at first sight that *QFEm.perg-3A* effect was only associated with the increment in GST, resulting in higher GN. The GST is usually not considered as a relevant trait

determining GN in semidwarf wheat under potential growing conditions because it used to be high, i.e., > 80% (Siddique et al. 1989; González et al. 2003; Elía et al. 2016), but nowadays it seems to be more relevant than expected. Using the two DH populations presented in Fig. 1, Pretini et al. (2020) showed a high correlation between GN and GST, and hence between FE and GST. Nevertheless, when *QFEm.perg-3A* pleiotropic effects were analyzed in detail, we could observe that the presence of the *QFEm.perg-3A* from B19 had a positive effect on the FFE (+ 2% FF − 3% SDW) and FE (+ 5% GN − 3% SDW), but it was 3.6- and 2.2-fold lower, respectively, than that of the 5A QTL. Then, although some of the effects of *QFEm.perg-3A* were not statistically significant at anthesis, our hypotheses is that the physiological processes taking place are similar to the ones described for *QFFE.perg-5A* (i.e., regulation of partitioning of assimilates within the spike at anthesis). But, as *QFEm.perg-3A* effect was lower at anthesis, and more associated with GST than FF, we detected it at maturity, when all the pleiotropic effects are accumulated (Fig. 4). It is noteworthy that almost no interaction was observed between both QTL for most of the measured variables, suggesting that their positive effects may be additive.

Genes within the corresponding regions (± 2 LOD away from the markers with the maximum peaks) were analyzed with annotated wheat reference genome IWGSC Ref. Seq. v1.0. The *QFEm.perg-3A* region contains 574 genes, and the *QFFE.perg-5A* region contains 1556 genes according to Appels et al. (2018). The contribution of a saturate genetic map in those regions would be necessary to identify candidate genes in future studies.

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Author contribution statement FGG identified the parental lines for developing the populations. FGG, IIT and AB generated the DH populations. IIT, JP, MG and MR helped with the initial genotyping and mapping of the populations. NP and LSV improved and set the final genetic map. IIT and FGG carried out the phenotyping experiments for 2012 and 2013. NP, IIT and FGG carried out the phenotyping experiments for 2015 and 2016. LSV, NP and FGG designed the validation experiments. NP and LSV conducted the F_2 genotyping and the QTL analyses. NP wrote the first manuscript with revision from LSV and FGG. FGG designed and coordinated the project.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest. This study does not include human or animal subjects.

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